FIG. 1
Context-Sensitive Parallel Optimization

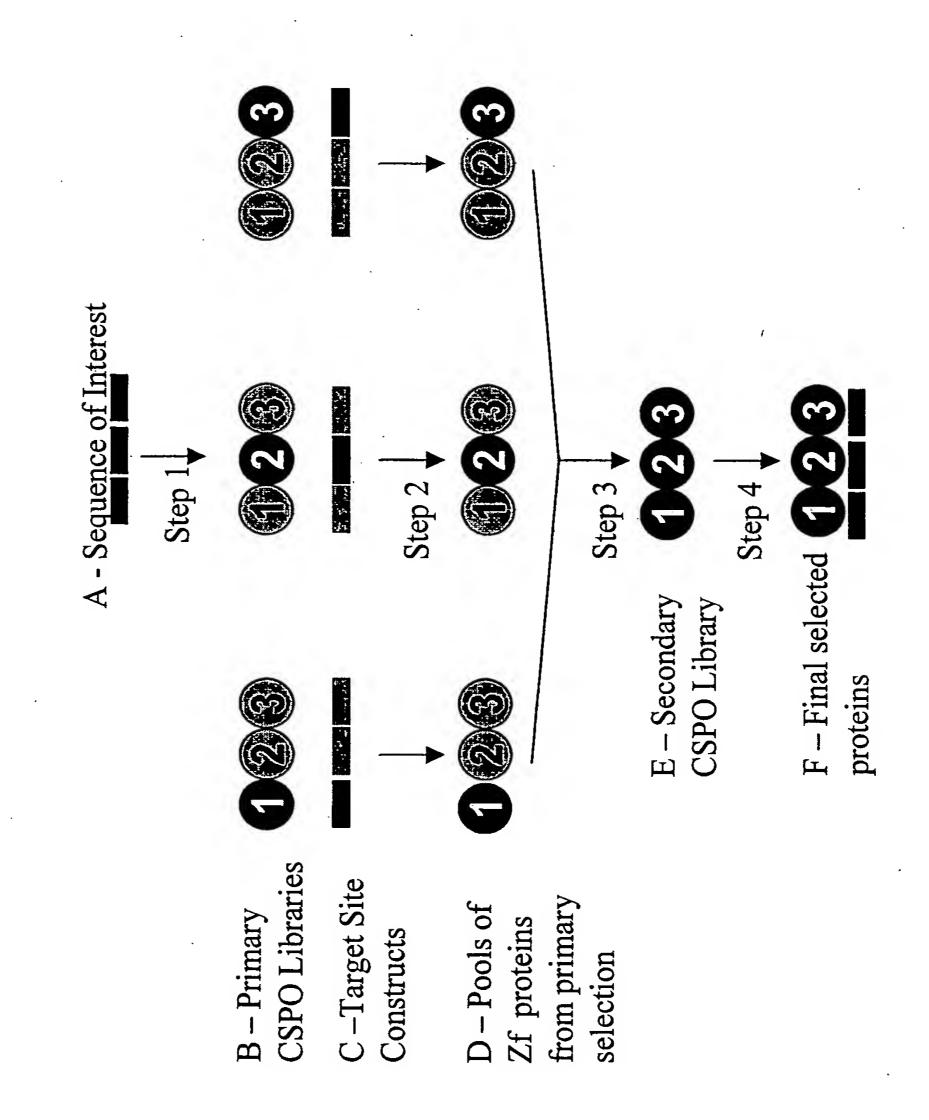


FIG. 2 Construction of Randomly Recombined Libraries

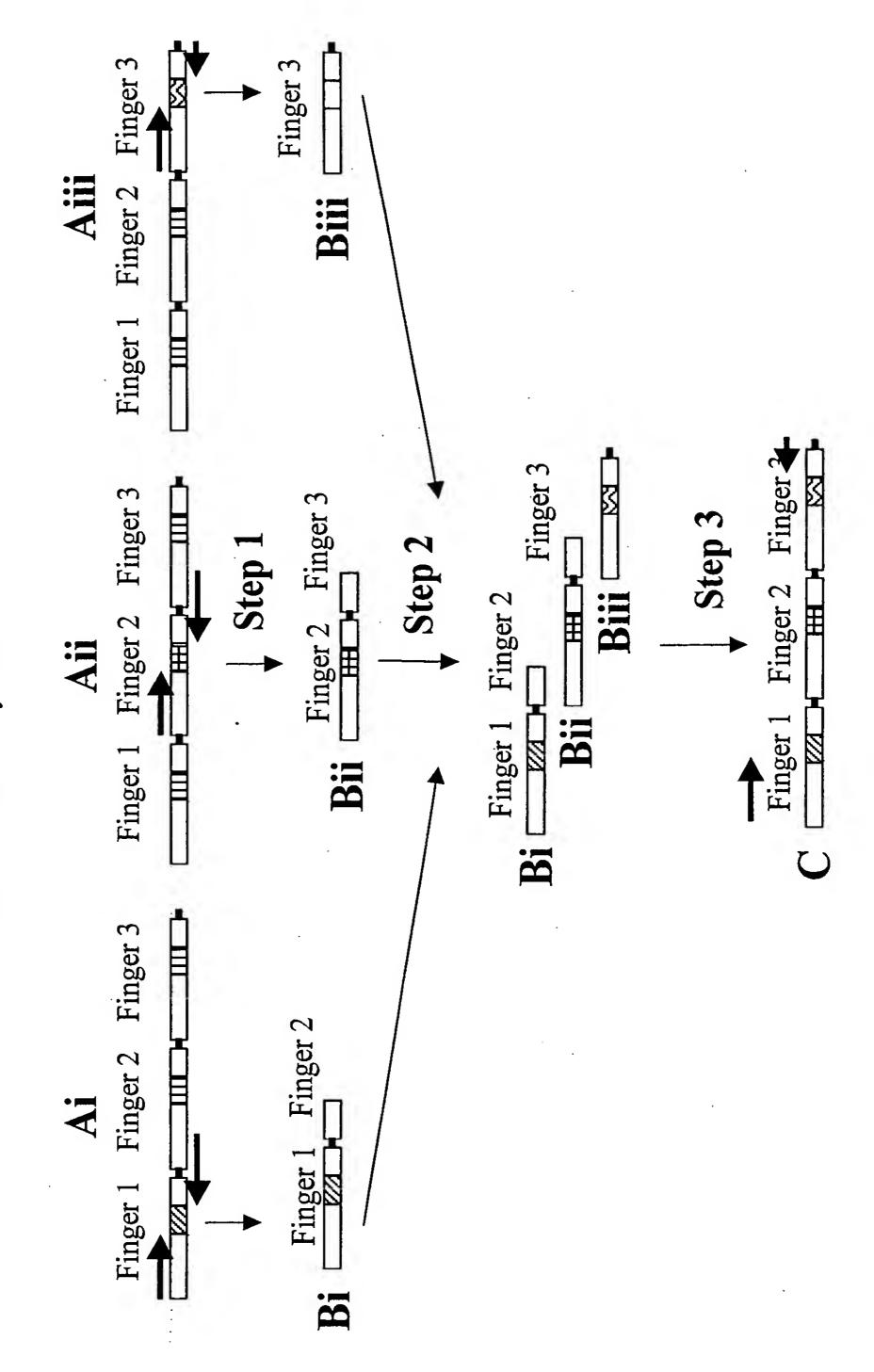


FIG. 3 Quantifying Affinity of ZFPs

Bound DNA

Free DNA

Bound DNA

Free DNA

B

A

A

B

A

Contact (HPB)

Contact (

FIG. 4
Characterizing Specificity of ZFPs  $\mathbf{m}$ A [Calf thymus DNA

FIG. 5

Validating Context-Sensitive Parallel Optimization

3CR-ABL

s'GCAGAAGCC3'

erb-B2

s'GCCGCAGTG3'

HIV promoter

5'GATGCTGCA3'

3CR-ABL

DRSSTR QGGNVR QAATQR\*

erb-B2

RKDSVR QSGDRR DCRDAR\*

HIV promoter

ASADTR NRSDSR TSSNKK#

fold-activation

FIG. 6
Selections for the BCR-ABL site

ACG ← DNA target site

1		-1 12356 -1 12356 -1 1235	1 12356 -	1 12356	
	"wild-type"	DRSSTR	DRSSTR QGGNVR QAATC	QAATQR	
	BCAB1	DSPTRR	DSPTRR QGANRR QANTÇ	QANTQR	
	BCAB2	DSPTRR	DSPTRR QNTNTR	QANTQR	
	BCAB3	DSPTRR	QNTNTR	QANTQR	
	BCAB4	DESTRR	DESTRR QGPNRR QRNTÇ	QRNTQR	
	BCAB5	DSPTRR	DSPTRR QGPNRR QGNTT	QGNTTR	
	BCAB6	DSPTRR	QNPNVR	QRNTQR	
	BCAB7	DSPTRR	DSPTRR QRTNIR QRNTÇ	QRNTQR	
	BCAB8	DRPTRR	DRPTRR QGANRR	QANTQR	
	BCAB9	DSPTRR	QNTINNR	QANTQR	
	BCAB10	DSPTRR	DSPTRR QKPNDR	QGNSIR	
	BCAB11	DSPTRR	DSPTRR QSTNNR QGNSN	QGNSNR	
~	BCAB12	DSPTRR	QNVNTR	QRNTQR	
₹ .					

FIG 7

## In vitro characterization of BCR-ABL ZFPs

Protein		Sequence	К <sub>а</sub> <sup>врес</sup> (рМ)	K <sub>d</sub> non-spec S	Specificity ratio	# of DNA bases specified
"wt"	DRSSTR	QGGNVR	DRSSTR QGGNVR QAATQR 28 (±3.9)	<b>55</b> (±12)	1,980	~5.5
BCAB 1	DSPTRR	QGANRR	<b>QANTQR 78</b> (±13)	2100 (±270)	27,000	~7.4
BCAB 7	DSPTRR	QRTNIR	QRNTQR 60 (±8.5)	1300 (±97)	23,000	~7.2
Zi£268			8.1(±1.8)	1000 (±120)	130,000	~8.5

FIG. 8
Selections for the erb-B2 site

GTG ACG CCG ← DNA target site

-1 12356 -1 12356 -1 12356

"wild-type"         RKDSVR           EB1         RQDIVK           EB2         RSDVAN           EB3         RSDVAN           EB4         RSDVAN           EB5         RSDVAN           EB6         RSDVAN           EB7         RSDLTK           EB9         RSDLTK           EB10         RQDIVK           EB11         RSDLTK           EB12         RSDLTK           EB11         RSDLTK           EB12         RPDVQK	OSGDRR OSSTTR	DCRDAR EKQGKR EKQGKR EKQGKR EKQGKR EKQGKR EKQGKR EKQGKR EKQGKR EKQGKR	
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FIG 9

In vitro characterization of erb-B2 ZFPs

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Protein	Sequence		K <sub>a</sub> spec ( <b>pM</b> )	K <sub>non-spec</sub> Sp (nM)	ecificity ratio	Specificity DNA bases ratio specified
"wt"	RKDSVR QSGDRR DCRDAR	DRR DCRDAR	150 (±23)	1000 (±120)	6,700	~6.4
EB 3	RSDVAN QSSTTR	TTR ERQGKR	31 (±3.1)	1100 (±15)	35,000	~7.5
EB 11	RSDLTK QSSTTR	TTR ERQGKR	65 (±3.9)	1100 (±81)	17,000	~7.0
Zi£268			8.1(±1.8)	1000 (±120)	130,000	~8.5

FIG. 10

Selections for the HIV promoter site

← DNA target site ACG TCG TAG + F1 F2 F3 -112356-112356 TÅG

		•		
"wild-type"	ASADTR	NRSDSR	TSSNKK	
HP1	LRADDN	LSQTKR	IRGNVR	
нр2	AKADDR	LSQTKR	VKSNRR	
нрз	LRADDR	LSQTKR	IGSNRR	
HP4	LRADDR	LSQTKR	VKSNRR	
нР5	LRTDDR	LRTDDR LSQTQR	LNSNAR	
нРб	LRTDDR	LSQTRR	LRSNGR	
не7	LRADDR	LSQTKR	MRSNMR	
нрв	LRADDR	LRADDR LRQTKR LRANLR	LRANLR	
нрэ	LRADDR	LAQTKR	IGSNTR	
HP10	LRTDDR	LRTDDR LSQTNR	LOGNKR	
HP11	LRADDR	LRADDR LRQTKR LRANLR	LRANLR	
HP12	NNAMVR	LSQTQR	MQGNSR	

fold-activation

FIG. 11 In vitro characterization of HIV Promoter ZFPs

Protein	Sequence	K <sub>d</sub> spec (pM)	K <sub>d</sub> non-spec Sr (nM)	Specificity ratio	# or DNA bases specified
"wt"	ASADTR NRSDSR	TSSNKK Unable	to calculate	(does not	not bind in vitro)
HP6	LRTDDR LSQTRR	LRSNGR 9.3 (±1.2)	2) 820 (±74)	87,000	~8.2
HP12	NNAMVR LSQTQR	AMVR LSQTQR MQGNSR 9.3 (±0.39)180	(8.8 <sup>±</sup> ) 180 (48.8)	19,000	~7.1
Zi£268		8.1(±1.8) 1000	3) 1000 (±120)	130,000	.8